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Facilitating kidney transplant tolerance

Leventhal *et al.*, *Sci Transl Med* 2012; **4**: 124ra28; doi:10.1126/scitranslmed.3003509

Currently, the most promising approach for human kidney transplant tolerance is the development of mixed chimerism within the kidney allograft recipient. Leventhal *et al.* used a nonmyeloablative conditioning approach, incorporating three pre-transplantation doses of fludarabine (30 mg/kg), two doses of cyclophosphamide (50 mg/kg) at day –3 and day +3, and low-dose total-body irradiation (200 Gy) in combination with an infusion of facilitating cells (FCs) to enhance the development of donor chimerism before weaning from immunosuppression. FCs were enriched from peripheral blood of granulocyte colony-stimulating factor-mobilized living kidney donors at least 2 weeks before transplantation, by a proprietary approach involving depletion of graft-versus-host disease (GVHD)-causing antigen-presenting cells. The FC fraction was given on day 1 after transplantation and comprised enriched hematopoietic stem cells and plasmacytoid precursor dendritic cells. Post-transplantation immunosuppression included tacrolimus and mycophenolate therapy. Of the eight patients treated, five developed 100% multilineage chimerism at 1 month and were successfully weaned from immunosuppressive therapy, with follow-up ranging from 6 to 20 months off. The conditioning regimen was well tolerated. In the weaned patient group, none developed engraftment syndrome secondary to the infusion, donor-specific alloantibody sensitization, or GVHD.

The authors performed extensive immunological monitoring of their transplant cohort, including identification of the presence of a significant number of $CD19^+$ B cells during the first year after transplantation. The precise mechanism whereby the FC fraction enhances chimerism development and allows donor-specific tolerance to develop is unclear, but preclinical studies in murine models suggest that they may induce antigen-specific T regulatory cells. Overall, this work marks another significant step toward the realization of kidney transplant tolerance in the clinic.
P. Toby Coates

Bicarbonate-containing peritoneal dialysates do not preferentially prevent reduction in residual renal function

Johnson *et al.*, *J Am Soc Nephrol* 2012; **23**: 1097–1107; doi:10.1681/ASN.2011121201

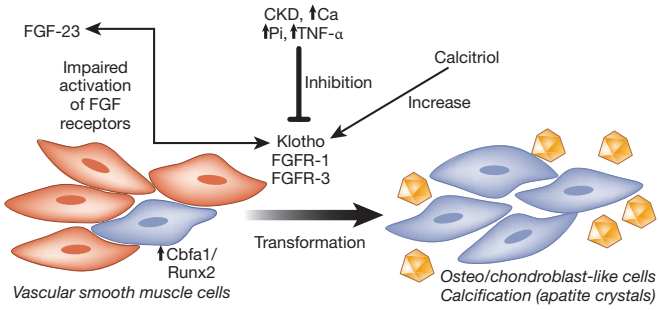
Bicarbonate-based dialysate is now the standard for hemodialysis, yet most peritoneal dialysis patients use lactate-containing fluids rather than bicarbonate. Several small prospective studies with limited follow-up have reported that neutral-pH bicarbonate-containing peritoneal dialysis fluids have had either a beneficial or a neutral effect on the preservation of residual renal function. Bicarbonate fluids are typically prepared in dual-chamber bags and, as such, contain less potentially nephrotoxic glucose degradation products following heat sterilization. However, these fluids are more expensive to produce and cost substantially more than standard lactate-containing peritoneal dialysis fluids. Johnson and colleagues conducted a randomized prospective trial of bicarbonate-containing compared with standard lactate-containing dialysates in incident patients starting peritoneal dialysis. Patient groups were well matched and had equal training and were followed for 24 months. There was no difference in the rate of loss of residual renal function, the primary outcome measure. However, on secondary analysis, the time to anuria was greater with bicarbonate-containing dialysates. Although the number of patients transferring to hemodialysis was similar in both groups, the time to the first episode of peritonitis was longer and the peritonitis rate lower with bicarbonate-containing dialysates. As daily ultrafiltration volumes were greater with standard peritoneal dialysates, the increased number of peritonitis episodes could have potentially led to episodes of relative hypovolemia with acute kidney injury, thus shortening the time to anuria. Alternatively, episodes of peritonitis may have been treated with nephrotoxic antibiotics, again risking acute kidney injury. The reported reduction in episodes of peritonitis remains to be explained.

This prospective multicenter trial does not support the use of more expensive neutral-pH bicarbonate-containing peritoneal dialysates to reduce the rate of loss of residual renal function. However, the reduction in the incidence of peritonitis episodes warrants further investigation, with further prospective trials specifically designed to examine peritonitis as a primary end point.
Andrew Davenport

Klotho deficiency and vascular disease in chronic kidney disease

Lim *et al.*, *Circulation* 2012; **125**: 2243–2255; doi:10.1161/CIRCULATIONAHA.111.053405

The role of Klotho (more precisely, α-Klotho) as an obligatory co-receptor for the activation of the fibroblast growth factor (FGF) receptors FGFR-1 and FGFR-3 by FGF-23 seemed firmly established, in line with the observation that the experimental deletion of either FGF-23 or Klotho leads to nearly identical phenotypes. However, recent doubt has arisen about the concept that Klotho and FGF-23 always have to work together. On the one hand, Klotho was shown to exert direct actions on the vessel wall in the absence of FGF-23. On the other, FGF-23 was found to have Klotho-independent actions on the myocardium, at least at high concentrations, such as those observed in the uremic state. Lim *et al.* examine the vascular



Tilman B. Drüeke

Klotho deficiency in CKD plays a major role in vascular calcification, partially via resistance to the action of fibroblast growth factor-23. Ca, calcium; Cbfa1, core-binding factor subunit α-1; CKD, chronic kidney disease; FGF-23, fibroblast growth factor-23; FGFR-1 and FGFR-3, fibroblast growth factor receptors 1 and 3; Pi, phosphate; Runx2, Runt-related transcription factor 2; TNF-α, tumor necrosis factor-α.

role of Klotho in more detail. First, they confirm the recent observation by Hu *et al.* that Klotho deficiency potentiates the development of human artery calcification. They further demonstrate expression of Klotho, FGFR-1, and FGFR-3 in human aortic smooth muscle cells (haSMCs), with downregulation in response to high concentrations of calcium and phosphate, tumor necrosis factor-α, and uremic serum in the incubation milieu, respectively. Klotho downregulation by these factors or inhibition of Klotho action by small interfering RNA (siRNA) enhances haSMC calcification. Notably, treatment with active vitamin D sterols of haSMCs maintained in a procalcific environment restores Klotho and FGFR expression and inhibits calcification. Finally, the physiological stimulation of haSMC survival and proliferation by FGF-23 is shown to be mitigated by Klotho siRNA, favoring haSMC transformation into osteoblast-like cells. Restoration of FGF-23 responsiveness and protection against haSMC phenotype change are achieved by full Klotho expression. In chronic kidney disease (CKD), kidney and parathyroid tissue Klotho content as well as circulating Klotho levels decrease progressively. Lim *et al.* now show that vessel wall Klotho and FGFRs also are decreased in CKD. Thus, Klotho appears to remain a major player in the CKD-associated mineral and bone disorder and cardiovascular disease (Figure).

Tilman B. Drüeke

Inducible podocyte injury and proteinuria in transgenic zebrafish

Zhou and Hildebrandt, *J Am Soc Nephrol* 2012; **23**: 1039–1047; doi:10.1681/ASN.2011080776

At present, the precise etiology of nephrotic syndrome remains unknown in a majority of cases. Podocytes are considered to play a major role in glomerular permselectivity, and their damage contributes to proteinuria. Mutations in podocyte-specific genes and soluble factors contributing to nephrotic syndrome have been identified in humans, but research is complicated by the lack of a simple, high-throughput test system. Zebrafish are useful for testing disease models, including for the kidney. Recently, Zhou and Hildebrandt have generated an inducible model of podocyte injury in zebrafish. Application of the prodrug metronidazole to the transgenic fish induces acute damage specifically to the podocytes, resulting in foot process effacement and podocyte loss. A functional assay for proteinuria was included by coexpression of green fluorescent protein (GFP)-tagged vitamin D-binding protein in the transgenic zebrafish. In the transgenic fish, induction of podocyte damage led to whole-body edema, and the proximal tubules reabsorbed and accumulated the GFP-tagged protein, mimicking the phenotype of human nephrotic syndrome.

Thus, this transgenic zebrafish model can be used as a simple high-throughput tool for studies of glomerular pathogenesis and podocyte regeneration.

Detlef Schlöndorff